CYTOLOGICAL OBSERVATIONS ON HYPOXIS:

I. SOMATIC CHROMOSOMES AND MEIOSIS IN SOME *HYPOXIS** SPECIES

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Abstract

Somatic chromosome numbers which varied from 16 to 96 were recorded for five "species" of Hypoxis. Meiosis was described in three "species" and in all three cases abnormalities (precocious chromosomes, laggards, multivalents and univalents) were recorded. These data, and the morphological variation which exists in this genus, suggest the occurrence of apomixis.

Hypoxis species are quite common in most parts of Southern Africa, and after superficial study, it seems quite easy to recognise different species e.g. *H. rooperi*, *H. nitida*, *H. rigidula* and others. As soon as one studies natural occurring populations more closely, however, one notices practically all intermediate stages and it becomes much more difficult—in fact in most cases impossible—to decide where the morphological variation of the one species stops and where the other begins. It is for example often impossible to decide whether a given specimen belongs to *H. rooperi* or *H. rigidula*, because one can see all the intermediate stages to both *H. rooperi* and *H. rigidula* in the same community. The taxonomist tries to solve this problem by looking for clear-cut differences, and in this way Verdoorn (1948—49) described *H. nitida*, using

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*After submitting this paper for publication the work of A. Fernandes and J. B. Neves (Sur la caryologie de quelques Monocotylédones africaines. Rendus IV^e Réunion A.E.T.F.A.T. 1961: 439-464) has come to my notice. They also studied *Hypoxis rooperi* and reported that the species did not have a homogenous chromosome number because they found plants with 76 and 114 somatic chromosomes. ($2n=76$ was comfirmed by $n=38$ determined in pollen mother cell meiosis and pollen mitosis.) The variation in chromosome number was neatly explained as being the result of different degrees of polyploidy (tetra- and hexaploidy with 19 as the basic number).

That $2n=76$, 96 and 114 have now been reported for this species does not surprise the present author. Not only are the plants most difficult to place into clear-cut species, but meiosis is abnormal and the plants are probably apomictic—in which case one can expect variation in chromosome number. I cannot accept the number $2n=114$ without query as such high counts can seldom be claimed as anything but approximations. It is surprising that the authors did not report any meiotic abnormalities in *H. rooperi*, as they were very common in my specimens.

the easily recognisable character of seed coat appearance—shiny or non-shiny. There is, however, no evidence that this is a specific difference—it may just be that two alleles are present in "shiny" and "non-shiny" and it is quite possible that the dominant prevails in some populations and is rare in other populations. The hairiness of the leaves (another character used to describe this species) varies greatly in populations.

Most of the differences are not due to environment, because the different morphological types commonly occur in the same habitat.

The cytological observations recorded in these three papers were made in order to gain some understanding of the species problem in Hypoxis. These studies include a description of somatic chromosomes (root tips) and of meiosis in the pollen-mother-cells, of the gametophytic cells (divisions in the pollen tubes) and also of embryo sac development. The results are submitted in three papers and should be regarded as being preliminary reports only, pointing the way to intensive future research.

The Somatic Chromosomes of some Species

Material and Methods

The specimens were identified by the staff of the National Herbarium in Pretoria, but in some cases only tentative identification could be obtained (e.g. *Hypoxis* cf. *zeyheri*).

If the root tips of any Hypoxis specimens are fixed in fixatives containing chromic acid they become overchromated within minutes. This blackening is so intense that subsequent staining is useless. Fixations with fluids without chromic acid (e.g. Carnoy's and FAA) did not give satisfactory results, and fixatives containing osmic acid resulted in intense blackening of tissues within seconds.

As the different Navashin fixatives are very reliable, it was decided to persist with Randolf's modified Navashin fluid (as quoted by Johansen, 1940) and to bleach the material after sectioning, but the sections could not be bleached in hydrogen peroxide nor chlorine solutions. The only successful method was to dip the slides in a 1% aqueous potassium permanganate solution for one minute, followed by a quick wash in water and a subsequent wash for 2 minutes in oxalic acid.

Dehydration of material was done in a graded series of ethyl and n-butyl alcohol, embedded in paraffin wax and the sections were 10—15 μ in thickness. Best staining results were obtained by using crystal violet, using the method of Smith (1934). The drawings were made by using a net micrometer in the eyepiece.

Somatic Chromosomes

Five species have been investigated.

(*a*) *Hypoxis stellipilis* (Ker.). Collected in the Alice (C.P.) district. Figure la. 2n = 16.

The chromosomes of Hypoxis species are generally small, but even so, the centromeres could clearly be seen in most cases. No secondary constrictions were recorded in any of the species. The chromosomes of *H. stellipilis* varied in length from 5 microns for the longest pair to under 2 microns for the shortest chromosome.

The centromeres are sub-median in 5 pairs, sub-terminal in the remaining 3.

(*b*) *Hypoxis zeyheri* (?) Baker. Figure lb. 2n = 32.

The material was collected at the Hogsback in the Alice district. The specimens were named tentatively only, because they differed from the type description of *H. zeyheri* by having hairy leaves.

The chromosomes are shorter than in *H. stellipilis*, and the longest chromosomes at full metaphase are under 4 microns in length. There are four pairs of very short chromosomes in which the centromeres could not be seen, in four pairs the centromeres are very close to median, and in the others the centromere position is sub-median to sub-terminal.

(*c*) *Hypoxis multiceps* Buchinge. 2n = 36. Figure 1c.

The chromosomes vary in length from 5 to more or less 1½ microns. The centromere position could not be determined for the shortest chromosomes. The chromosomes were too numerous and small to enable one to recognise the different homologous pairs.

(*d*) *Hypoxis longifolia* Baker. 2n = 72. Figure 1d.

The material was collected in the East London district.

Centromere positions could be recorded in most cases (Fig. 1d) but the genome is too complex for further analysis. Chromosome length varied from over 6 to under 2 microns.

(*e*) *Hypoxis rooperi* Moore. 2n = 96. Figure l e.

The material was collected close to East London. There are so many chromosomes, and so little variation in length that karyotype analyses becomes almost meaningless. Even the number recorded (96) must be considered as tentative only.

It is obvious from the above results that polyploidy has played an important role in speciation of these plants, but from the above recorded chromosome numbers it is impossible to suggest a basic number. Eight can be considered as the basic number for four of the species, but it cannot explain the 2n = 36, which occurs in *H. multiceps*.

MEIOSIS IN THE ANTHERS OF HYPOXIS SPECIES.

Meiosis occurs before the inflorescences appear, and the plants produce

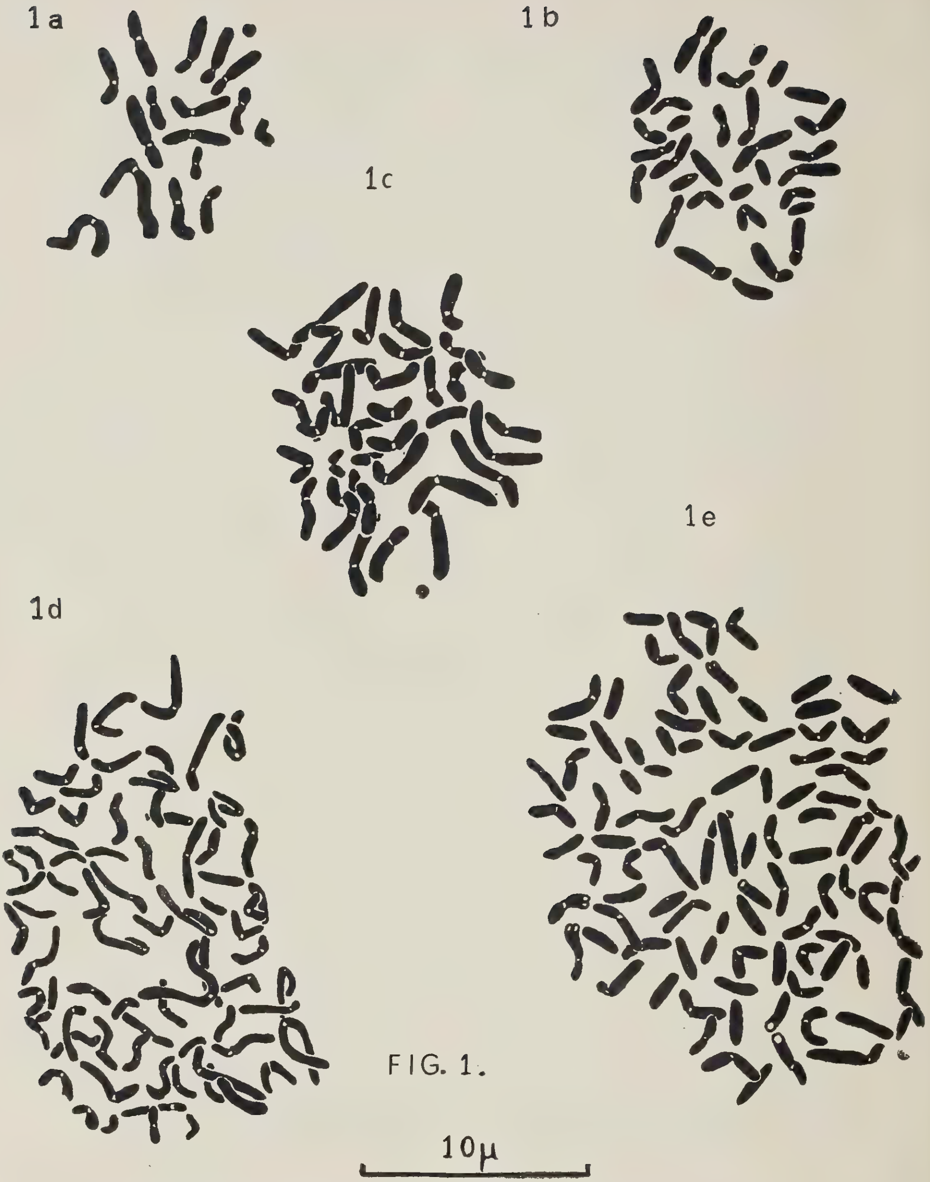

FIG. 1.

FIGURE 1: Metaphase plates in root tips of different Hypoxis species.
Fig. 1a: H. stellipilis, 2n = 16. Fig. 1b: H. zeyheri, 2n = 32.
Fig. 1c: H. multiceps, 2n = 36. Fig. 1d: H. longifolia, 2n = 72.
Fig. 1e: H. rooperi, 2n = 96.

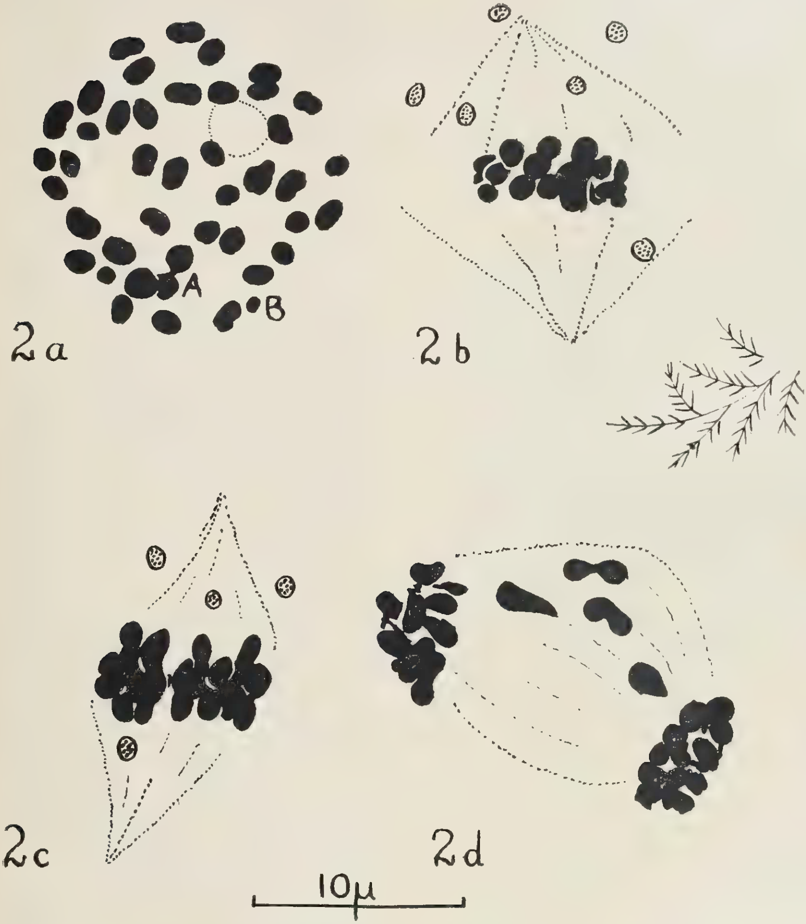

FIGURE 2: Meiosis in pollen mother cells of *Hypoxis nitida*.
Fig. 2a: Diakinesis, n = ± 40—42. A—multivalent; B—univalent.
Fig. 2b: Metaphase I, with precocious univalents.
Fig. 2c: Metaphase I, with precocious univalents.
Fig. 2d: Late anaphase I, with laggards.

several inflorescences. Young flowers can be obtained when the first inflorescences appear by gripping the leaves firmly and pulling, which causes a break at the base of the leaves. Between these soft leaf bases several young inflorescences can be collected for meiotic studies.

The anthers were removed, smeared on a clean slide and immediately fixed and stained in aceto-carmine. The preparations were made semi-permanent by sealing the edges of the coverslip with nail varnish, and such slides were stored at plus or minus 4°C.

Results:

1. *Hypoxis nitida:* n = ±40—42.

In Fig. 2a (which represents diakinesis) one can see three bivalents which are associated with the nucleolus. *A* represents a multivalent and some small chromosome bodies, *B* may represent a univalent.

At metaphase some chromosomes, which are interpreted as univalents, move precociously (Figs. 2b, 2c) and at this stage they do not stain as deeply as the chromosome associations on the equatorial plate.

At anaphase laggards are practically always present. (Fig. 2d).

It is extremely difficult to determine the haploid chromosome number, but it appears to be between 40 and 42.

2. *Hypoxis rooperi* (Witwatersrand).

FIRST MEIOTIC DIVISION

Diakinesis:

Chromosome associations are here often so complicated that analysis of diakinesis fails—see e.g. at the configurations at E in Figs. 3a and 3b.

Multivalents are common e.g. a multivalent of 5 at *A* in Fig. 3b, of four at *B* in Fig. 3a, of 3 at *C* in Fig. 3b. Univalents also occur e.g. *D* in Fig. 3a.

The multivalents labelled E in Fig. 3a and 3b consist of more than 5 chromosomes.

Metaphase:

Most of the chromosome associations become orientated on the metaphase plate, but some univalents move precociously (A in Fig. 3c).

Late Anaphase:

When most of the chromosomes have reached the poles, several laggards are still present between the chromosome groups (see Fig. 3d and 3e). Some of the laggards consist of associations of 2 chromosomes (A in Fig. 3e), but in some cases multivalent associations lag behind the others, e.g. the lagging multivalent of 6 chromosomes in Fig. 3d.

Telophase:

Some of the laggards are not included when the new nuclei are formed (Fig. 3f).

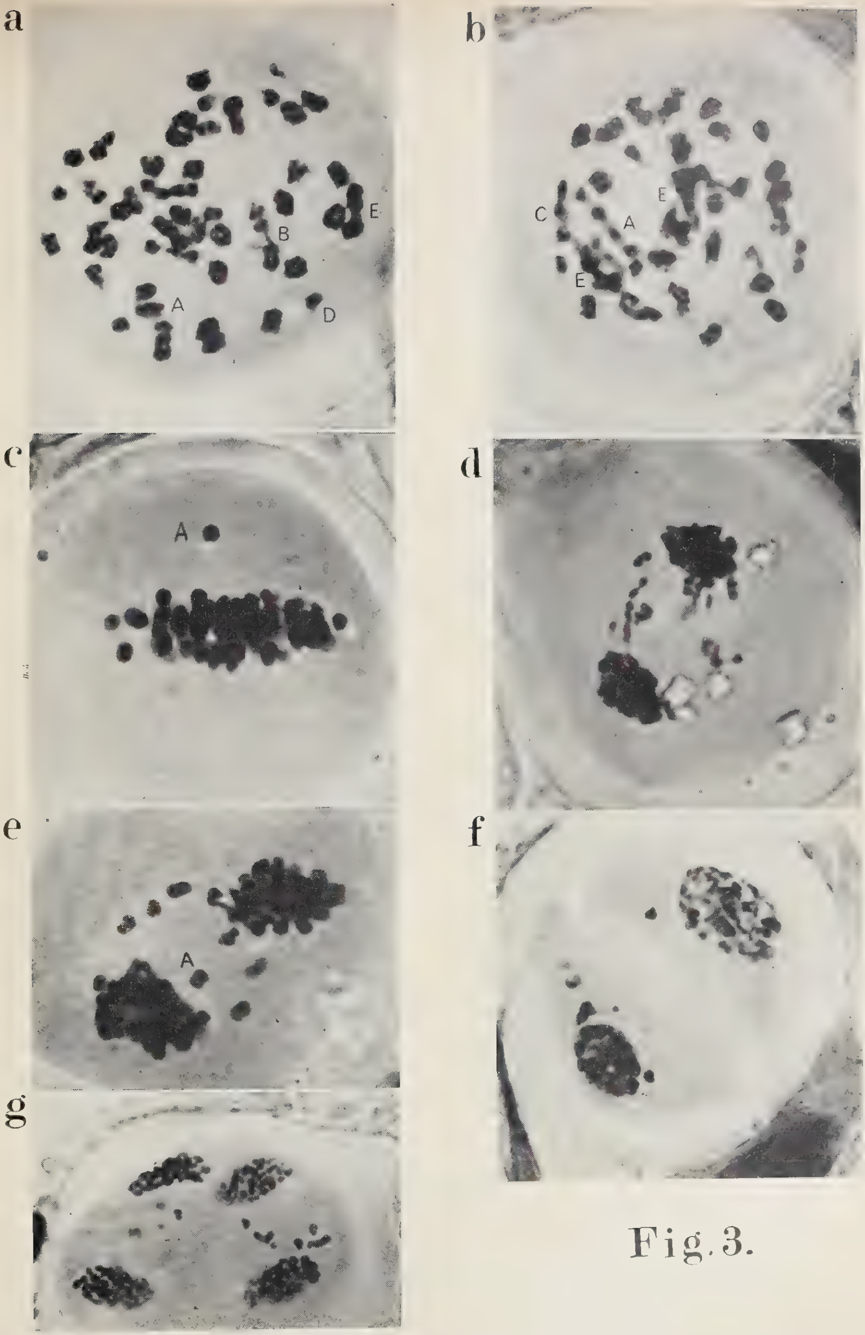

Fig.3.

FIGURE 3: Meiosis in pollen mother cells of *Hypoxis rooperi*.

Figures 3a and 3b: Diakinesis. A: multivalent of 5 chromosomes; B: multivalent of 4 chromosomes; C: trivalent; D: univalent; E: complicated multivalents of more than 5 chromosomes.

Fig. 3c: Metaphase I with precocious univalents.

Fig. 3d: Late anaphase I, with laggards—one laggard of six chromosomes shows clearly.

Fig. 3e: Late anaphase I, with lagging bivalents.

Fig. 3f: Laggards being excluded from nuclei formed at telophase I.

Fig. 3g: Laggards being excluded from nuclei at telophase II.

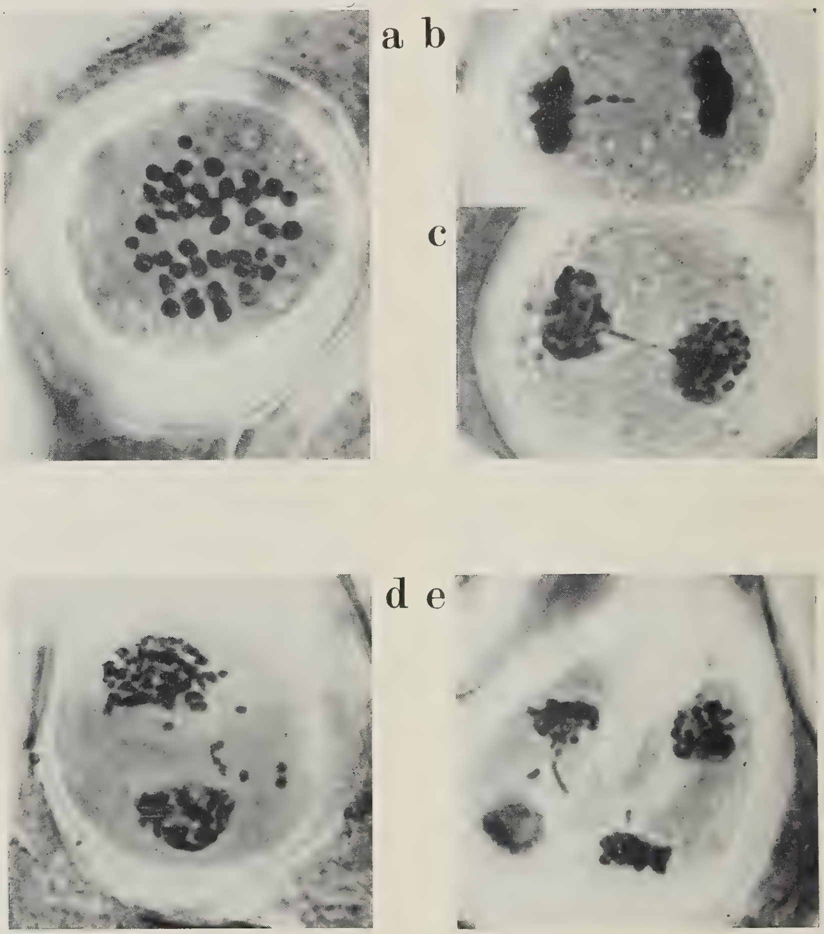

Fig. 4.

FIGURE 4: *Hypoxis species.*

Fig. 4a: Diakinesis.
Figs. 4b and 4c: Showing a lagging multivalent and a bridge at telophase I.
Fig. 4d: Chromosomes are excluded from nuclei at telophase I.
Fig. 4e: Laggard at telophase II.

SECOND MEIOTIC DIVISION

Several chromosomes are excluded from the nuclei which are formed at the second telophase (see Fig. 3g).

These abnormalities were recorded in *Hypoxis rooperi* specimens collected at Honeydew (District Roodepoort), but similar abnormalities were also seen in plants collected at Mondeor (District Johannesburg).

3. *Hypoxis species:*

Fig. 4a represents diakinesis, and although multivalents and univalents are present the haploid chromosome number is more or less 40.

Precocious chromosomes are present at metaphase I, bridges and laggards are present at anaphase I and telophase I (Fig. 4b and 4c) and laggards are also present at telophase II (Fig. 4e). Some laggards are excluded from the nuclei that are formed at the end of meiosis I (Fig. 4d).

At metaphase I, 33% of the divisions were abnormal, at anaphase I, 46% and at anaphase II, 48%.

Discussion

It is clear that polyploidy has played an important role during speciation in the genus *Hypoxis*, but from the results presented here it is impossible to determine the basic chromosome number. The basic chromosome number is most likely below 9 because at least one species has 16 somatic chromosomes.

There are such numerous abnormalities during meiosis of the high chromosome-number species that one would expect a high degree of sterility if the plants were reproducing sexually. This is not the case, because the capsules have numerous healthy seeds.

The high polyploidy, abnormal meiosis and morphological variation (which occurs within a species) are indicative of apomixis occurring in this genus. If apomixis occurs, then pollination is still necessary because in several experiments when flowers were emasculated and bagged no further development of the ovules took place.

If these plants are apomictic, all the seeds from one plant will develop into similar individuals, whereas if hybridization is very common (which could also explain the morphological variation and abnormal meiosis) great variation will be seen in such offspring. In several experiments in our department in which seeds were planted, not a single one germinated although several conventional methods of overcoming seed dormancy were tried (e.g. alternate freezing and thawing, alternate periods of light and dark, chipping and abrazing the testa, soaking seed in H_2SO_4). After trying these methods to germinate seed a paper by Markotter (1936) came to our notice, in which he germinated seed of the closely related genera *Forbesia*, *Pauridia* and *Ianthe*, and in which he demonstrated that these seeds required an after-ripening period of a year. This would explain the negative results obtained in our department.

This long after-ripening period makes progeny-testing time consuming. The hypothesis that several *Hypoxis* species are apomictic was tested by embryosac analysis. This will be reported in a later paper.

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